Refine Search

Search Results -

Terms	Documents
L2 and ctla4	3

Database:

US Pre-Grant Publication Full-Text Database
US Patents Full-Text Database
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JPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search: L3

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Recall Text Clear Interrupt

Search History

DATE: Thursday, October 19, 2006 **Purge Queries** Printable Copy Create Case

Set Name side by side	**Query**	**Hit Count**	**Set Name** result set
	DB=USPT; PLUR=YES; OP=AND		
L3	L2 and ctla4	3	L3
L2	L1 and hybridoma	79	L2
L1	human adj sequence adj antibod?	79	L1

END OF SEARCH HISTORY

Lonberg; Nils	Woodside	CA	US
Deo; Yashwant M.	Annandale	NJ	US
Keler; Tibor P.	Ottsville	PA	US

US-CL-CURRENT: 530/388.22; 530/387.1, 530/387.9, 530/388.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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3. Document ID: US 6479258 B1

L3: Entry 3 of 3 File: USPT Nov 12, 2002

US-PAT-NO: 6479258
DOCUMENT-IDENTIFIER: US 6479258 B1
**** See image for Certificate of Correction ****

TITLE: Non-stochastic generation of genetic vaccines

DATE-ISSUED: November 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Rancho Santa Fe	CA		

US-CL-CURRENT: 435/69.1; 530/350, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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Terms	Documents
L2 and ctla4	3

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Search Results - Record(s) 1 through 3 of 3 returned.

1. Document ID: US 7041870 B2

L3: Entry 1 of 3 File: USPT May 9, 2006

US-PAT-NO: 7041870
DOCUMENT-IDENTIFIER: US 7041870 B2

TITLE: Transgenic transchromosomal rodents for making human antibodies

DATE-ISSUED: May 9, 2006

PRIOR-PUBLICATION:

DOC-ID	DATE
US 20020199213 A1	December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tomizuka; Kazuma	Takasaki			JP
Ishida; Isao	Kanagawa			JP
Lonberg; Nils	Woodside	CA		US
Halk; Edward L.	Sunnyvale	CA		US

US-CL-CURRENT: 800/13; 800/14, 800/15, 800/16, 800/17, 800/18, 800/19, 800/20, 800/21, 800/22, 800/23, 800/24, 800/25

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

2. Document ID: US 6984720 B1

L3: Entry 2 of 3 File: USPT Jan 10, 2006

US-PAT-NO: 6984720
DOCUMENT-IDENTIFIER: US 6984720 B1

TITLE: Human CTLA-4 antibodies

DATE-ISSUED: January 10, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Korman; Alan J.	Piedmont	CA		US
Halk; Edward L.	Sunnyvale	CA		US

First Hit Fwd Refs Previous Doc Next Doc Go to Doc#
End of Result Set

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L1: Entry 79 of 79 File: USPT Apr 29, 1997

DOCUMENT-IDENTIFIER: US 5625126 A
TITLE: Transgenic non-human animals for producing heterologous antibodies

Detailed Description Text (173):
In some variations, it may be desirable to produce a trans-switched immunoglobulin. For example, such trans-switched heavy chains can be chimeric (i.e., a non-murine (human) variable region and a murine constant region). Antibodies comprising such chimeric trans-switched immunoglobulins can be used for a variety of applications where it is desirable to have a non-human (e.g., murine) constant region (e.g., for retention of effector functions in the host, for the presence of murine immunological determinants such as for binding of a secondary antibody which does not bind human constant regions). For one example, a human variable region repertoire may possess advantages as compared to the murine variable region repertoire with respect to certain antigens. Presumably the human V.sub.H, D, J.sub.H, V.sub.L, and J.sub.L genes have been selected for during evolution for their ability to encode immunoglobulins that bind certain evolutionarily important antigens; antigens which provided evolutionary selective pressure for the murine repertoire can be distinct from those antigens which provided evolutionary pressure to shape the human repertoire. Other repertoire adavantages may exist, making the human variable region repertoire advantageous when combined with a murine constant region (e.g., a trans-switched murine) isotype. The presence of a murine constant region can afford advantages over a human constant region. For example, a murine .gamma. constant region linked to a human variable region by trans-switching may provide an antibody which possesses murine effector functions (e.g., ADCC, murine complement fixation) so that such a chimeric antibody (preferably monoclonal) which is reactive with a predetermined antigen (e.g., human IL-2 receptor) may be tested in a mouse disease model, such as a mouse model of graft-versus-host disease wherein the T lymphocytes in the mouse express a functional human IL-2 receptor. Subsequently, the human variable region encoding sequence may be isolated (e.g., by PCR amplification or cDNA cloning from the source (hybridoma clone)) and spliced to a sequence encoding a desired human constant region to encode a human sequence antibody more suitable for human therapeutic uses where immunogenicity is preferably minimized. The polynucleotide(s) having the resultant fully human encoding sequence(s) can be expressed in a host cell (e.g., from an expression vector in a mammalian cell) and purified for pharmaceutical formulation. For some applications, the chimeric antibodies may be used directly without replacing the murine constant region with a human constant region. Other variations and uses of trans-switched chimeric antibodies will be evident to those of skill in the art.

Detailed Description Text (186):
In a variation, hybridoma clones producing antibodies having high binding affinity (e.g., at least 1.times.10.sup.7 M.sup.-1, preferably at least 1.times.10.sup.8 M.sup.-1, more preferably at least 1.times.10.sup.9 M.sup.-1 or greater) are obtained by selecting, from a pool of hybridoma cells derived from B cells of transgenic mice harboring a human heavy chain transgene capable of isotype switching (see, supra) and substantially lacking endogenous murine heavy chain loci capable of undergoing productive (in-frame) V-D-J rearrangement, hybridomas which

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L1: Entry 66 of 79 File: USPT Jul 3, 2001

US-PAT-NO: 6255458
DOCUMENT-IDENTIFIER: US 6255458 B1

TITLE: High affinity human antibodies and human antibodies against digoxin

DATE-ISSUED: July 3, 2001

not relied

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lonberg; Nils	Woodside	CA		
Kay; Robert M.	San Francisco	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
GenPharm International	San Jose	CA			02

APPL-NO: 09/042353 [PALM]
DATE FILED: March 13, 1998

PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 08/758,417 filed Dec. 2, 1996, which is a continuation-in-part of U.S. Ser. No. 08/728,463 filed Oct. 10, 1996, which is a continuation-in-part of U.S. Ser. No. 08/544,404 filed Oct. 10, 1995, now U.S. Pat. No. 5,770,429, which is a continuation-in-part of U.S. Ser. No. 08/352,322 filed Dec. 7, 1994 now U.S. Pat. No. 5,625,126, which is a continuation-in-part of U.S. Ser. No. 08/209,741 filed Mar. 9, 1994, now abandoned which is a continuation-in-part of U.S. Ser. No. 08/165,699 filed Dec. 10, 1993, now abandoned which is a continuation-in-part of U.S. Ser. No. 08/161,739 filed Dec. 3, 1993, now abandoned which is a continuation-in-part of U.S. Ser. No. 08/155,301 filed Nov. 18, 1993 now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/096,762 filed Jul. 22, 1993 now U.S. Pat. No. 5,814,318, which is a continuation-in-part of U.S. Ser. No. 08/053,131 filed Apr. 26, 1993, now U.S. Pat. No. 5,661,016 which is a continuation-in-part of U.S. Ser. No. 07/990,860 filed Dec. 16, 1992 now U.S. Pat. No. 5,545,806 which is a continuation-in-part of U.S. Ser. No. 07/904,068 filed Jun. 23, 1992 now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/853,408 filed Mar. 18, 1992 now U.S. Pat. No. 5,789,650, which is a continuation-in-part of U.S. Ser. No. 07/834,539, filed Feb. 5, 1992, now U.S. Pat. No. 5,633,425, which is a continuation-in-part of U.S. Ser. No. 07/810,279 filed Dec. 17, 1991 now U.S. Pat. No. 5,569,825 which is a continuation-in-part of U.S. Ser. No. 07/575,962 filed Aug. 31, 1990 now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/574,748 filed Aug. 29, 1990 now abandoned. This application also claims priority benefits under Title 35, United States Code, Section 120, to PCT Application No. PCT/US91/06185, filed Aug. 28, 1991, (which corresponds to U.S. Ser. No. 07/834,539 filed Feb. 5, 1992), PCT Application No. PCT/US92/10983, filed Dec. 17, 1992 PCT Application No. PCT/US94/04580, filed Apr. 25, 1994 PCT Application No. PCT/US96/16433, filed Oct. 10, 1996, and PCT Application No. PCT/US97/21803, filed Dec. 1, 1997.

INT-CL-ISSUED: [07] C07K 16/00

INT-CL-CURRENT:

TYPE	IPC	DATE
CIPS	A01 K 67/027	20060101
CIPS	C07 K 14/435	20060101
CIPS	C12 N 15/11	20060101
CIPS	C12 N 15/00	20060101
CIPS	C12 N 15/87	20060101
CIPS	C12 N 5/16	20060101
CIPS	C12 N 15/85	20060101
CIPS	C12 N 15/90	20060101
CIPN	A61 K 38/00	20060101
CIPS	C07 K 16/46	20060101
CIPS	C07 K 14/725	20060101
CIPS	C07 K 16/00	20060101
CIPS	C07 K 16/30	20060101
CIPS	C07 K 16/18	20060101
CIPS	C07 K 16/28	20060101
CIPS	C07 K 16/44	20060101
CIPS	C07 K 16/42	20060101

US-CL-ISSUED: 530/388.15; 530/388.9, 435/326
US-CL-CURRENT: 530/388.15; 435/326, 530/388.9

FIELD-OF-CLASSIFICATION-SEARCH: 424/175.1, 435/326, 435/346, 435/345, 530/388.9, 530/388.15
See application file for complete search history.

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
5567610	October 1996	Borrebaeck et al.	

OTHER PUBLICATIONS

Sawada et al. Bul. Natl. Inst. Hyg. Sci. vol. 9108, pp. 29-33, 1990.*
Woolf et al. New Engl. J. Med. vol. 326, pp. 1739-1744, 1992.*
Fishwild, et al. Nature Biotechnology. vol. 14, pp. 845-851, 1996.

ART-UNIT: 164

PRIMARY-EXAMINER: Chan; Christina Y.

ASSISTANT-EXAMINER: DiBrino; Marianne

ATTY-AGENT-FIRM: Townsend and Townsend and Crew LLP

ABSTRACT:

The invention relates to transgenic non-human animals capable of producing heterologous antibodies and methods for producing human sequence antibodies which bind to human antigens with substantial affinity.

2 Claims, 119 Drawing figures

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L3: Entry 2 of 3 File: USPT Jan 10, 2006

US-PAT-NO: 6984720
DOCUMENT-IDENTIFIER: US 6984720 B1

TITLE: Human CTLA-4 antibodies

DATE-ISSUED: January 10, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Korman; Alan J.	Piedmont	CA		US
Halk; Edward L.	Sunnyvale	CA		US
Lonberg; Nils	Woodside	CA		US
Deo; Yashwant M.	Annandale	NJ		US
Keler; Tibor P.	Ottsville	PA		US

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Medarex, Inc.	Annandale	NJ		US	02

APPL-NO: 09/644668 [PALM]
DATE FILED: August 24, 2000

102(e)

RELATED-US-APPL-DATA:
us-provisional-application US 60150452 00 19990824

INT-CL-ISSUED:

TYPE	IPC	DATE	IPC-OLD
IPCP	C12P21/08	20060101	C12P021/08
IPCS	C07K16/00	20060101	C07K016/00
IPCS	C07K16/28	20060101	C07K016/28

INT-CL-CURRENT:

TYPE	IPC	DATE
CIPS	C07 K 16/00	20060101
CIPP	C12 P 21/08	20060101
CIPS	C07 K 16/28	20060101

US-CL-ISSUED: 530/388.22; 530/387.1, 530/387.9, 530/388.1
US-CL-CURRENT: 530/388.22; 530/387.1, 530/387.9, 530/388.1

FIELD-OF-CLASSIFICATION-SEARCH: 530/387.1, 530/387.9, 530/388.1, 530/388.22, 530/388.15, 530/388.2, 530/388.7, 530/388.73, 530/388.75, 530/387.3
See application file for complete search history.

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
4399216	August 1983	Axel et al.	
4681581	July 1987	Coates	
4683195	July 1987	Mullis et al.	
4683202	July 1987	Mullis	
4735210	April 1988	Goldenberg	
4740461	April 1988	Kaufman	
4816397	March 1989	Boss et al.	
4921040	May 1990	Ueruenduel et al.	
4959455	September 1990	Clark et al.	
5101827	April 1992	Goldenberg	
5151510	September 1992	Stec et al.	
5194594	March 1993	Khawli et al.	
5434131	July 1995	Linsley et al.	
5530101	June 1996	Queen et al.	
5545806	August 1996	Lonberg et al.	
5545807	August 1996	Surani et al.	
5569825	October 1996	Lonberg et al.	
5585089	December 1996	Queen et al.	
5591669	January 1997	Krimpenfort et al.	
5612205	March 1997	Kay et al.	
5625126	April 1997	Lonberg et al.	
RE35500	May 1997	Rhodes	
5633425	May 1997	Lonberg et al.	
5643763	July 1997	Dunn et al.	
5648471	July 1997	Buttram et al.	
5661016	August 1997	Lonberg et al.	
5693761	December 1997	Queen et al.	
5693792	December 1997	Torii et al.	
5697902	December 1997	Goldenberg	
5703057	December 1997	Johnston et al.	
5714350	February 1998	Co et al.	
5721367	February 1998	Kay et al.	

5733743	March 1998	Johnson et al.	
5741957	April 1998	Deboer et al.	
5750172	May 1998	Meade et al.	
5756687	May 1998	Denman et al.	
5770197	June 1998	Linsley et al.	
5770429	June 1998	Lonberg et al.	
5773253	June 1998	Linsley et al.	
5777085	July 1998	Co et al.	
5789215	August 1998	Berns et al.	
5789650	August 1998	Lonberg et al.	
5811097	September 1998	Allison et al.	
5814318	September 1998	Lonberg et al.	
5827690	October 1998	Meade et al.	
5844095	December 1998	Linsley et al.	
5855887	January 1999	Allison et al.	
5874299	February 1999	Lonberg et al.	
5877397	March 1999	Lonberg et al.	
5885796	March 1999	Linsley et al.	
5916771	June 1999	Hori et al.	
5939598	August 1999	Kucherlapati et al.	
5968510	October 1999	Linsley et al.	
5977318	November 1999	Linsley et al.	
6051227	April 2000	Allison et al.	
6075181	June 2000	Kucherlapati et al.	
6114598	September 2000	Kucherlapati et al.	
6150584	November 2000	Kucherlapati et al.	
6162963	December 2000	Kucherlapati et al.	
6207156	March 2001	Kuchroo et al.	
6255458	July 2001	Lonberg et al.	
6682736	January 2004	Hanson et al.	424/144.1
2002/0086014	July 2002	Korman et al.	

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	CLASS
2205680	November 1998	CA	
0 256 055	August 1991	EP	

US-CL-CURRENT: 800/13; 800/14, 800/15, 800/16, 800/17, 800/18, 800/19, 800/20, 800/21, 800/22, 800/23, 800/24, 800/25

FIELD-OF-CLASSIFICATION-SEARCH: 800/13-25
See application file for complete search history.

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
5175384	December 1992	Krimpenfort	
5204244	April 1993	Fell et al.	
5416260	May 1995	Koller	
5434340	July 1995	Krimpenfort	
5545806	August 1996	Lonberg et al.	
5545807	August 1996	Surani	
5569825	October 1996	Lonberg et al.	
5625126	April 1997	Lonberg et al.	
5633425	May 1997	Lonberg et al.	
5661016	August 1997	Lonberg et al.	
5698196	December 1997	Matsushima	
5702946	December 1997	Doerschuk	
5770429	June 1998	Lonberg et al.	
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6300129	October 2001	Lonberg et al.	
6632976	October 2003	Tomizuka et al.	800/18
2003/0093820	May 2003	Green et al.	800/8

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	CLASS
0 315 062	May 1989	EP	
0 773 288	May 1997	EP	

0 843 961	May 1998	EP
0 972 445	January 2000	EP
1 106 061	June 2000	EP
1 206 906	May 2002	EP
WO 90/04036	April 1990	WO
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ART-UNIT: 1632

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ABSTRACT:

The present invention provides novel transgenic nonhuman mammals capable of producing human sequence antibodies, as well as methods of producing and using these antibodies.

4 Claims, 14 Drawing figures

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0 338 841	March 1995	EP
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0 546 073	September 1997	EP
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ART-UNIT: 1644

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ATTY-AGENT-FIRM: Darby & Darby

ABSTRACT:

The present invention provides human sequence antibodies against CTLA-4 and methods of treating human diseases, infections and other conditions using these antibodies.

14 Claims, 23 Drawing figures

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L3: Entry 1 of 3 File: USPT May 9, 2006

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Brief Summary Text (4):
Antibodies represent a class of therapeutic molecules with applications in many different areas including transplantation, cardiovascular diseases, infectious diseases, cancer, and autoimmunity (Goldenberg, M., 1999, Clin. Ther. 21:309 318; Present, D. et al., 1999, New Engl. J. Med. 340:1398 1405; Targan, S. et al., 1997, New Engl. J. Med. 337:1029 1035; Davis, T. et al., 1999, Blood 94:88a; Saez-Llorens, X. et al., 1998, Pediatr. Infect. Dis. J. 17:787 791; Berard, J. et al., 1999, Pharmacotherapy 19:1127 1137; Glennie, M. et al. 2000, Immunol. Today 21:403 410; Miller, R., 1982, New Engl. J. Med. 306:517 522; Maini, R., et al., 1999, Lancet, 354:1932 1939). The development of hybridoma technology enabled the isolation of rodent monoclonal antibodies (also referred to as MAbs) as candidate therapeutic molecules (Kohler, G. and Milstein, C., 1975, Nature 256:495 497). However, early studies involving the use of non-human monoclonal antibodies for in vivo human therapy, demonstrated that human anti-mouse antibody (HAMA) responses could limit the use of such agents (Schroff, R. et al., 1985, Cancer Res. 45,879 885; Shawler, D. et al., 1985, J. Immunol. 135:1530 1535). Thus it is recognized that a reduction in the immunogenicity of therapeutic antibodies is desirable. Recombinant DNA technologies have been employed to reduce the immunogenicity of non-human antibodies (Boulianne, G. et al., 1984, Nature 312, 643 646; Morrison, S. et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851 6855; Riechmann, L. et al., 1988, Nature 332:323 327; Jones, P. et al., 1986, Nature 321:522 525; Queen, C. et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:10029 10033). However, it is also recognized that fully human monoclonal antibodies are a potential source of low immunogenicity therapeutic 30 agents for treating human diseases (Little, M. et al., 2000, Immunol. Today 21:364 70). The use of transgenic mice carrying human immunoglobulin (Ig) loci in their germline configuration provide for the isolation of high affinity fully human monoclonal antibodies directed against a variety of targets including human self antigens for which the normal human immune system is tolerant (Lonberg, N. et al., 1994, Nature 368:856 9; Green, L. et al., 1994, Nature Genet. 7:13 21; Green, L. & Jakobovits, 1998, Exp. Med. 188:483 95; Lonberg, N and Huszar, D., 1995, Int. Rev. Immunol. 13:65 93; Bruggemann, M. et al., 1991, Eur. J. Immunol. 21:1323 1326; Fishwild, D. et al., 1996, Nat. Biotechnol. 14:845 851; Mendez, M. et al., 1997, Nat. Genet. 15:146 156; Green, L., 1999, J. Immunol. Methods 231:11 23; Yang, X. et al., 1999, Cancer Res. 59:1236 1243; Bruggemann, M. and Taussig, M J., Curr. Opin. Biotechnol. 8:455 458, 1997). Human antibodies fall into a variety of different classes based on light chain (kappa and Lambda) and heavy chain (IgA.sub.1, IgA.sub.2, IgD, IgE, IgG.sub.1, IgG.sub.2, IgG.sub.3, IgG.sub.4, and IgM). These different classes potentially provide for different therapeutic uses. For example, the different heavy chain isotypes have different interactions with complement and with cell based Fc receptors. Some of the heavy chain classes (IgM and IgA) can also form multimers, thus increasing the valency of F.sub.c and V region interactions. It is therefore desirable to have a platform for

generating human monoclonal antibodies of all isotypes. However, the large size of human Ig loci (1 2 Mb) had been a major obstacle for the introduction of entire loci into transgenic mice to reconstitute full diverse human antibody repertoires because the cloning of over megabase-sized DNA fragments encompassing whole human Ig loci was difficult even with the use of yeast artificial chromosomes. Recently, a novel procedure using a human chromosome itself as a vector for transgenesis facilitated the transfer of the complete IgH and IgK loci into transgenic mice without the need for cloning DNA fragments into artificial DNA vectors (Tomizuka, K. et al., 1997, Nature Genet. 16:133 143; Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. 97:722 727). Tomizuka et al. (Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722 727) demonstrated the introduction of two transmittable human chromosome fragments (hCFs), one containing the immunoglobulin (Ig) heavy chain locus (IgH, .about.1.5 Mb) and the other the K light chain locus (IgH, .about.2 Mb), into a transgenic mouse strain whose endogenous IgH and IgK loci were inactivated. In the resultant double-transchromosomic (Tc)/double-knockout (KO) mice, a substantial proportion of the somatic cells retained both hCFs, and the rescue in the defect of Ig production was showed by high level expression of human Ig heavy and kappa light chains in the absence of mouse heavy and kappa light chains. In addition, serum expression profiles of four human Ig .gamma. subclasses resembled those seen in humans. The transgenic mice developed an antigen-specific human antibody response upon immunization with human serum albumin (HSA), and HSA-specific human monoclonal antibodies with various isotypes were obtained from them. The study of Tomizuka et al. (ibid.) also demonstrated the instability of hChr.2-derived hCF containing the Igk locus (hCF(2-W23)) in mice. The observed instability of the .kappa. transchromosome could be a impediment to optimal human kappa light chain expression and production of human kappa-positive hybridomas. Indeed, two-thirds of anti-HSA hybridomas obtained from a double-Tc/KO mouse were mouse lambda-positive (m.lamda..sup.+) and a majority (83%) of IgG/mX hybridomas was found to have lost the hCF(2-W23). Therefore, there is a need for transgenic animals that retain characteristics conferred by the transchromosomes described by Tomizuka et al. (ibid.), particularly animals that express substantially the full repertoire of human heavy chain isotypes, and also exhibit improved stability of introduced human sequences, allowing for increased efficiency of obtaining fully human antibodies.

Brief Summary Text (8):
The invention further provides methods for generating a plurality of B cells expressing human antibody sequences, the method comprising: providing the transgenic nonhuman mammal comprising two human immunoglobulin loci, wherein one of two said human immunoglobulin loci is a human heavy chain locus and the other locus is a human light chain locus; and wherein only one of said loci is of a transchromosome, and immunizing the transgenic nonhuman mammal to generate a plurality of B cells expressing human antibody sequences. In some such methods, the transchromosome is a fragment of human chromosome 14. In some such methods, the human transchromosome is human chromosome fragment SC20 (hCF(SC20)). Some such methods further comprise collecting the plurality of B cells expressing sequences expressing human antibodies. Some such methods further comprise fusing the plurality of B cells with immortalized cells to form hybridomas. Other such methods further comprise collecting the human antibody sequences from the hybridomas. In some such methods, the human antibody sequences are purified. Some such methods further comprise collecting the sequences encoding human antibodies. In some such methods the sequences encoding human antibodies are full length. In some methods, the sequences encoding human antibodies are expressed in transfected cells. In some such methods, the human light chain locus comprises unrearranged sequences from the natural human kappa light chain locus. In some such methods, the human kappa light chain locus is the inserted KCo5 transgene. In some such methods, the plurality of B cells comprises at least a first B cell encoding an antibody with a first isotype selected from the group consisting of IgA, IgD, IgE, IgG and IgM. In some methods the IgA isotype is IgA.sub.1 or IgA.sub.2. In some methods the IgG isotype is IgG.sub.1, IgG.sub.2, IgG.sub.3 or IgG.sub.4. In some such methods, the plurality of B cells further comprises at least a second B cell encoding an antibody with a

second isotype different from the first isotype selected from the group consisting of IgA, IgD, IgE, IgG and IgM. In some methods, the plurality of B cells comprise at least five B cells each encoding an antibody having a different isotype wherein the isotypes of the antibodies are IgA, IgD, IgE, IgG and IgM respectively. In another aspect, the transgenic nonhuman mammal further comprises a mutation of a gene, wherein the mutation increases the immune response to autoantigen. In some such methods, the mutation is the inactivation of the Fc-gamma IIB gene.

Brief Summary Text (9):
The invention further provides a method for generating a human sequence antibody that binds to a predetermined antigen, the method comprising the following steps: immunizing a transgenic nonhuman mammal with a predetermined antigen, wherein the transgenic nonhuman mammal comprises two human immunoglobulin loci, wherein one of two said human immunoglobulin loci is a human heavy chain locus and the other locus is a human light chain locus; and wherein only one of said loci is of a transchromosome; and collecting the human sequence antibody from the immunized nonhuman mammal. In another aspect, the transgenic nonhuman mammal further comprises a mutation of a gene, wherein the mutation increases the immune response to autoantigen. In some such methods, the mutation is the inactivation of the Fc-gamma IIB gene.

Brief Summary Text (11):
The invention further provides a method for generating antigen-specific hybridomas secreting human sequence antibody, the method comprising: immunizing the transgenic nonhuman mammal with a predetermined antigen, wherein the transgenic nonhuman mammal comprises two human immunoglobulin loci, wherein one of two said human immunoglobulin loci is a human heavy chain locus and the other locus is a human light chain locus; and wherein only one of said loci is of a transchromosome; fusing lymphocytes from the transgenic nonhuman mammal with immortalized cells to form hybridoma cells; and determining the binding of the antibody produced by the hybridoma cells to the predetermined antigen. In some such methods greater than 50% of the antigen-specific hybridoma clones secrete antibody having human heavy chain and human light chain. In another aspect, the transgenic nonhuman mammal further comprises a mutation of a gene, wherein the mutation increases the immune response to autoantigen. In some such methods, the mutation is the inactivation of the Fc-gamma IIB gene.

Brief Summary Text (12):
The invention further provides a method for generating a human sequence antibody that binds to a predetermined antigen, the method comprising the following steps: immunizing a transgenic nonhuman mammal with a predetermined antigen; wherein the transgenic nonhuman mammal comprises two human immunoglobulin loci, wherein one of two said human immunoglobulin loci is a human heavy chain locus and the other locus is a human light chain locus wherein only one locus is of a transchromosome; and screening hybridoma cells formed for the presence of antigen reactive antibodies. In some such methods, the hybridoma cells are subcloned at an efficiency of greater than 20%. In some such methods, the antigen reactive antibodies are secreted from the hybridoma in culture. In some such methods, the antigen reactive antibodies are substantially pure. In some methods, the substantially pure antibodies are formulated for therapeutic use. In another aspect, the transgenic nonhuman mammal further comprises a mutation of a gene, wherein the mutation increases the immune response to autoantigen. In some such methods, the mutation is the inactivation of the Fc-gamma IIB gene.

Brief Summary Text (16):
The invention further provides a method for generating a human sequence antibody, or fragment thereof, that binds to a predetermined antigen, the method comprising the following steps: immunizing a transgenic nonhuman mammal with a predetermined antigen, wherein the transgenic nonhuman mammal comprises two human immunoglobulin loci, wherein one of two said human immunoglobulin loci is a human heavy chain

locus and the other locus is a human light chain locus wherein only one locus is of a transchromosome; collecting antibody V region sequences from the immunized transgenic nonhuman mammal; cloning the collected V regions into a DNA vector generating an expression library; expressing the library to identify V region sequences that encode an antibody, or fragment thereof, that binds to the predetermined antigen. In another aspect, the transgenic nonhuman mammal further comprises a mutation of a gene, wherein the mutation increases the immune response to autoantigen. In some such methods, the mutation is the inactivation of the Fc-gamma IIB gene.

Brief Summary Text (17):
The invention further provides a method for generating a human sequence antibody or fragment thereof, that binds to a predetermined antigen, the method comprising the following steps: immunizing a transgenic nonhuman mammal with a predetermined antigen, wherein the transgenic nonhuman mammal comprises at least two human immunoglobulin loci, wherein one of said human immunoglobulin loci is a human heavy chain locus and the other locus is a human light chain locus; and wherein at least one locus is of a transchromosome; isolating CDNA coding at least one human antibody V region from B cells of the immunized transgenic nonhuman mammal or from hybridomas generated by fusion of said B cell and an immortalized cell; cloning said CDNA into an expression vector; introducing said vector into a host cell; culturing said host cell; and collecting said human sequence antibody or fragment thereof from said host cell or culture medium thereof. In some such methods, the isolating step is performed by PCR. In some such methods, the isolating step is performed by cDNA library screening using at least one DNA probe. In some such methods the isolating step is performed by phage display library screening. In some such methods, the CDNA encodes full length human antibody sequences. In some methods, the collected human sequence antibody isotype is different from the isotype of antibody producing cells of said immunized transgenic nonhuman mammal. In another aspect, the transgenic nonhuman mammal further comprises a mutation of a gene, wherein the mutation increases the immune response to autoantigen. In some such methods, the mutation is the inactivation of the Fc-gamma IIB gene.

Brief Summary Text (18):
The invention further provides a method of improving the stability of a transchromosomic mouse hybridoma cell expressing a human antibody reactive with a predetermined antigen, the method comprising: breeding a first mouse, the first mouse comprising a first human immunoglobulin locus on a transchromosome, together with a second mouse, the second mouse comprising a second human immunoglobulin locus inserted within an endogenous mouse chromosome; obtaining a third mouse from the breeding, the third mouse comprising both the first and the second human immunoglobulin loci; immunizing the third mouse, or its progeny, with the predetermined antigen; collecting B cells from the immunized mouse; and fusing the B cells with immortalized cells to obtain hybridoma cells expressing the human antibody reactive with the predetermined antigen. Some such methods further comprise: culturing the hybridoma cells in media; testing the media to identify the presence of hybridoma cells that express human antibodies reactive with the predetermined antigen; diluting the hybridoma cells; and culturing the diluted hybridoma cells to obtain clonal cell lines expressing a monoclonal human antibody reactive with the predetermined antigen. In some such methods, the clonal cell lines are obtained from at least 50% of the identified hybridoma cells.

Brief Summary Text (19):
In another aspect, the invention provides a mouse hybridoma cell secreting a human sequence antibody having an IgA isotype that binds to a specified antigen with an equilibrium association constant (Ka) of at least 10.sup.10 M.sup.-1.

Brief Summary Text (20):
In another aspect, the invention provides a human sequence antibody having an IgA isotype that binds to a specified antigen with an equilibrium association constant

(K.sub.a) of at least 10.sup.10 M.sup.-1.

Description Paragraph (8):
FIG. 7. Growth curve and anti-CD4 human monoclonal antibody production of the KM2-3 hybridoma cells.

Description Paragraph (18):
The human sequence antibodies of the invention can be produced in a non-human transgenic mammal, e.g., a transgenic mouse, capable of producing multiple isotypes of human (e.g., monoclonal or polyclonal) antibodies (e.g., IgM, IgD, IgG, IgA and/or IgE) to a variety of antigens by undergoing V-D-J recombination and, for non IgM/non IgD antibodies, isotype switching. Accordingly, various aspects of the invention include antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as non-human transgenic mammals, and B-cells and hybridomas for making such monoclonal antibodies.

Description Paragraph (24):
The terms "cytotoxic T lymphocyte-associated antigen-4," "CTLA-4," "CTLA4," "CTLA-4 antigen" and "CD152" (see, e.g., Murata, 1999, Am. J. Pathol. 155:453 460) are used interchangeably, and include variants, isoforms, species homologs of human CTLA-4, and analogs having at least one common epitope with CTLA-4 (see, e.g., Balzano, 1992, Int. J. Cancer Suppl. 7:28 32).

Description Paragraph (28):
The term "human sequence antibody" includes antibodies having variable and constant regions (if present) derived from human immunoglobulin sequences. The human sequence antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human sequence antibody", as used herein, is not intended to include antibodies in which entire CDR sequences sufficient to confer antigen specificity and derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., humanized antibodies).

Description Paragraph (29):
The terms "monoclonal antibody" or "monoclonal antibody composition" refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions (if present) derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

Description Paragraph (94):
To achieve improved stability of the human kappa light chain locus, the trans-chromosome technology was combined with earlier pronuclear microinjection technology for generating trangenic animals. The human kappa light chain locus transgene KCo5 (Fishwild, D. et al., 1996, Nat. Biotechnol. 14:845 851; U.S. Pat. No. 5,770,429) includes a substantial portion of the human kappa locus, and is stably maintained in the mouse germline and in B cells and hybridoma cells expressing human kappa chains derived from the transgene. This transgene was combined with the stable hCF(SC20) transchromosome, together with functional inactivation mutations of the endogenous mouse heavy and kappa light chain loci, to generate animals expressing a broad human antibody repertoire including multiple human heavy chain isotypes. Thus, improved stability of the light chain transgene, relative to the double-TC/KO mice (Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722 727) provides for the recovery of a larger number of hybridomas

from each fusion.

Description Paragraph (107):
Production of Monoclonal Antibodies by Hybridoma Fusion

Description Paragraph (108):
The production of monoclonal antibodies can be accomplished by, for example, immunizing the animal with an antigen (e.g., a human protein antigen such as CD4, G-CSF, HSA, EGFR, or CTLA-4, a pathogen encoded antigen, a toxin, or other antigen). A longer polypeptide comprising the antigen or an immunogenic fragment of the antigen or anti-idiotypic antibodies to an antibody to the antigen can also be used. See Harlow & Lane, Antibodies, A Laboratory Manual (CSHP New York, N.Y., 1988) and Mishell and Shiigi, Selected Methods in Cellular Immunology, (W.H. Freeman and Co. New York, N.Y. 1980) (both references are incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptide synthesis or by recombinant expression. Optionally, the immunogen can be administered attached or otherwise complexed with a carrier protein, as described below. Optionally, the immunogen can be administered with an adjuvant. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Rodents (e.g., mice, rats, and hamsters) are typically used for making monoclonal antibodies. These mice can be transgenic, and can comprise human immunoglobulin gene sequences, as described below. After immunization, the immunized animals will develop a serum response to the introduced immunogen. This serum response can be measured by titration of collected serum using a variety of different assays. An example of a commonly used assay is an ELISA. The magnitude of the serum response is commonly referred to as the titer. For example, a titer of 1,000 indicates that the presence of reactive antibodies can be detected by assay of a 1,000 fold dilution of the serum. Typically, immunization will result in a serum response several orders of magnitude greater than that found in unimmunized animals. Serum responses of only one or two orders of magnitude are considered weak, and typically indicate the presence of few B cells expressing antigen reactive antibodies. Monoclonal antibodies are routinely obtained by fusing lymphocytes with immortalized cells (e.g., myeloma cells) to form hybrid cells, referred to as hybridoma cells. The newly formed hybridoma cells derive antibody expression properties from the parental lymphocytes, and growth properties from the parental immortalized cells. Newly formed hybridoma cells are grown in culture dishes (e.g., 96 well plates) comprising culture medium. The culture supernatant is tested (typically between one and two weeks after fusion) for the presence of antigen reactive antibodies of the desired heavy and light chain isotype. The cells in this primary culture are then diluted and replated to isolate individual clones of hybridoma cells secreting a single species of monoclonal antibody. This secondary culture can be further subcloned to obtain tertiary cultures, and so forth. The fraction of antigen reactive primary cultures that can be used to obtain hybridoma clones by this process of subcloning provides a measure of the subcloning efficiency. If all of the antigen positive primary hybridoma cultures can be used to derive cloned cell lines, then the subcloning efficiency is 100%. If the immunoglobulin loci that encode the expressed antibodies are unstable, e.g., easily lost during cell division--either through loss of a chromosome, chromosome fragment, or transchromosome, or through deletional recombination of an inserted array, or through some other mechanism--then the subcloning efficiency will be reduced (i.e., less than 100%). It is particularly useful to have a platform for deriving monoclonal antibodies where the subcloning efficiency is high (e.g., greater than 20%, preferably greater than 50%, more preferably greater than 80%, most preferably greater than 90% or 95%). Antibodies are screened for specific binding to the antigen. Optionally, antibodies are further screened for binding to a specific region of the antigen. For protein antigens, the latter screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of a the antigen peptide and determining which deletion mutants bind to the

antibody. Binding can be assessed, for example, by Western blot or ELISA. The smallest fragment to show specific binding to the antibody defines the epitope of the antibody. However, some epitopes comprise non-contiguous structural elements that can be lost by deletion of elements outside of the actual epitope. Alternatively, epitope specificity can be determined by a competition assay is which a test and reference antibody compete for binding to the antigen. If the test and reference antibodies compete, then they bind to the same epitope or epitopes sufficiently proximal that binding of one antibody interferes with binding of the other.

Description Paragraph (109):
Cloning Nucleic Acids Encoding Antibodies From B Cells Hybridomas

Description Paragraph (110):
Nucleic acids encoding at least the variable regions of heavy and light chains can be cloned from either immunized or naive transgenic animals. Nucleic acids can be cloned as genomic or cDNA from lymphatic cells of such animals. No immortalization of such cells is required prior to cloning of immunoglobulin sequences. Usually, mRNA is isolated and amplified by reverse transcription with oligo-dT primers. The cDNA is then amplified using primers to conserved regions of human immunoglobulins. The libraries can be easily enriched for non-mu isotypes using a 3' primer specific for non-mu sequences (e.g., IgG or IgA) Typically, the amplified population of light chains comprises at least 100, 1000, 10,000, 100,000 or 1,000,000 different light chains. Likewise, the amplified population of heavy chains comprises at least 100, 1000, 10,000, 100,000 or 1,000,000 different heavy chains. For example, using IgG primers, typically at least 90, 95 or 99% of amplified heavy chains are of IgG isotype. Nucleic acids encoding at least the variable regions of heavy and light chains can also be cloned from hybridomas mentioned above, by various well-known methods such as PCR or screening cDNA library by DNA probe specific for conserved regions of human antibodies. Nucleic acids encoding antibody chains to be subcloned can be excised by restriction digestion of flanking sequences or can be amplified by PCR using primers to sites flanking the coding sequences. See generally PCR Technology: Principles and Applications for DNA Amplification (ed. H. A. Erlich, Freeman Press, NY, N.Y., 1992); PCR Protocols: A Guide to Methods and Applications (eds. Innis, et al., Academic Press, San Diego, Calif., 1990); Mattila, et al., 1991, Nucleic Acids Res. 19:967; Eckert, et al., 1991, PCR Methods and Applications 1: 17; PCR (eds. McPherson et al., IRL Press, Oxford). These references and references cited therein are herein incorporated by reference for all purposes.

Description Paragraph (126):
The basic approach and an exemplary cell fusion partner, SPAZ-4, for use in this approach have been described by Oestberg et al., 1983, Hybridoma 2:361 367; Oestberg, U.S. Pat. No. 4,634,664; and Engleman et al., U.S. Pat. No. 4,634,666 (each of which is incorporated by reference in its entirety for all purposes). The antibody-producing cell lines obtained by this method are called triomas, because they are descended from three cells--two human and one mouse. Initially, a mouse myeloma line is fused with a human B-lymphocyte to obtain a non-antibody-producing xenogeneic hybrid cell, such as the SPAZ-4 cell line described by Oestberg, supra. The xenogeneic cell is then fused with an immunized human B-lymphocyte to obtain an antibody-producing trioma cell line. Triomas have been found to produce antibody more stably than ordinary hybridomas made from human cells.

Description Paragraph (139):
The nucleotide sequences of heavy and light chain transcripts from a hybridomas are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa light chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol.

Chem. 266:19867 19870); and, HindIII sites are engineered upstream of the translation initiation sites.

Description Paragraph (162):
The invention provides pharmaceutical compositions comprising one or a combination of monoclonal antibodies (intact or binding fragments thereof) formulated together with a pharmaceutically acceptable carrier. Some compositions include a combination of multiple (e.g., two or more) monoclonal antibodies or antigen-binding portions thereof of the invention. In some compositions, each of the antibodies or antigen-binding portions thereof of the composition is a monoclonal antibody or a human sequence antibody that binds to a distinct, pre-selected epitope of an antigen.

Description Paragraph (202):
DNA sequence analysis of CDNA clones derived directly from the KCo5 double transgenic/double deletion mice, or from hybridomas generated from these animals, revealed expression of the following V kappa genes: L6, A27, O12, O4/O14, A10, L15, L18, L19, and L24.

Description Paragraph (208):
Serum samples prepared from 6 12 week old cross-bred mice were examined by ELISAs to determine concentrations of human Ig, .mu., .gamma., .kappa. and mouse .lamda. chains (FIG. 2). Compared with the mice hemizygous for endogenous C.mu. deletion, kept under similar conditions, the average levels of human Ig .mu. and Ig .gamma. were higher than mouse .mu., chain level (273 mg/l) and one third of the mouse .gamma. chain level (590 mg/l), respectively. These heavy chain expression levels are similar to those of double-Tc/double-KO mice (hCF(SC20)/hCF(2-W23)/CM2D/CKD Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci U.S.A. 97:722 727). One fourth of F2 offspring produced by mating between male and female cross-bred mice were expected to be homozygous for the m.lamda.C1 (.lamda.low) mutation because the first generation of cross-bred mice were heterozygous for this mutation. Serum concentrations of human Ig .kappa. and mouse Ig .lamda. light chains were determined by ELISA in twenty one F2 cross-bred mice as described in the previous report (Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722 727). Of 21 mice examined, six mice exhibited low (<0.1) mouse .lamda./human .kappa. ratio, which is characteristics of mice homozygous for the .lamda. low mutation (Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722 727). Thus, these six cross-bred mice may be homozygous for the .lamda.(low) mutation, which can be useful for efficient production of hybridomas that secrete antibodies comprising human Ig heavy and .kappa. light chains.

Description Paragraph (213):
Generation of hybridomas. Splenocytes from immunized mice were fused to Sp2/0-Ag14 cells on day 40. The cell suspension were inoculated into 384-well plates at 20 thousands of splenocytes per well. Resulting hybridomas were screened for production of monoclonal antibodies (MAbs) to sCD4. The results are shown below in Table 1.

Description Paragraph (215):
The parental hybridomas from cross-bred mouse were subcloned by two rounds of limiting dilution with high efficiency. All of hybridomas from cross-bred mouse secreted human .gamma./human .kappa. anti-CD4 MAbs and none of hybridomas secreted human .gamma./murine .kappa. anti-CD4 MAbs. These data indicated that cross-bred mouse is superior to the double TC/KO strain for generation of antigen-specific human monoclonal antibodies. The isotype of the MAbs secreted by these subcloned hybridomas was further examined by a number of ELISAs. Seven wells were h.gamma.1.sup.+ and 7 wells were h.gamma.4.sup.+.

Description Paragraph (216):
Growth Curve and Secretion Levels for an Anti-CD4Human IgG.sub.1 Monoclonal Antibody in Small Scale Cultures. One of the hybridoma clones producing anti-CD4

human IgG.sub.1.kappa. (KM2-3) was used for the determination of growth curve and secretion levels for the human monoclonal antibody in small scale cultures. KM2-3 hybridoma cells were seeded in 4 liter spinner flask (Bellco) at 1.times.10.sup.5 cells/ml on day 0. One liter of ERDF medium supplied with ITS-X (Gibco BRL) and 1% low IgG serum (Hyclone) was used for culture. One ml of medium was collected every day, and the cell number and IgG.sub.1.kappa. concentration was measured by ELI SA as described in the previous report (Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722 727). The results were presented in FIG. 7. Estimated production rate was 24.6 pg/cell/day, which is within a range similar to that expected for excellent murine hybridomas under these conditions.

Description Paragraph (221):
Productions of hybridomas. Splenocytes from immunized mice were fused to Sp2/0-Ag14 cells on day 40 and resulting hybridomas were screened by ELISA for production of monoclonal antibodies (MAbs) to G-CSF. The results are shown below in Table 2.

Description Paragraph (223):
Half of anti-G-CSF IgG producing hybridomas secreted human .gamma./human .kappa. anti-G-CSF MAbs and remaining of hybridomas secreted human .gamma./murine .kappa. anti-G-CSF MAbs. Hybridomas producing h.gamma./h.kappa. antibodies were subcloned by two rounds of limiting dilution. Further ELISA experiments demonstrated that 5, 3, and 3 wells were h.gamma.1.sup.+, h.gamma.2.sup.+, and h.gamma.4.sup.+, respectively.

Description Paragraph (227):
Generation of hybridomas. Splenocytes from immunized mice were fused to Sp2/0-Ag14 cells on day 24 and resulting hybridomas were screened by ELISA for production of monoclonal antibodies (MAbs) to antigen. Ten wells of hybridomas were chosen randomly from anti-albumin h.gamma. producing hybridomas and subcloned. All of hybridomas secreted human .gamma./human .kappa. anti-albumin. This data indicate that cross-bred mouse is superior to double-Tc/double-KO mouse for production of antigen-specific fully human monoclonal antibodies since two-thirds of anti-albumin IgG hybridomas obtained from double-Tc/double-KO mouse were m.lamda..sup.+ (Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722 727).

Description Paragraph (230):
Antigen. A DNA segment encoding a fusion protein comprising sequences from the human CTLA-4 and the murine CD3.zeta. genes was constructed by PCR amplification of cDNA clones together with bridging synthetic oligonucleotides. The encoded fusion protein contains the following sequences: i.) human CTLA-4 encoding amino acids 1 190 (containing the signal peptide, the extracellular domain of human CTLA-4 and the entirety of the presumed transmembrane sequence of human CTLA-4) and ii.) murine CD3.zeta. from amino acid 52 to the carboxy terminus. The amplified PCR product was cloned into a plasmid vector and the DNA sequence was determined. The cloned insert was then subcloned into the vector pBABE (which contains a gene encoding for puromycin resistance (Morganstem, JP and Land, H, 1990 Nucl. Acids Res. 18:3587 96) to create pBABE-huCTLA-4/CD3.zeta.. pBABE-huCTLA-4/CD3.zeta. was transfected into the retroviral packaging line, .psi.-2, and a pool of puromycin resistant cells were selected. These cells were co-cultured with the murine T cell hybridoma BW5147 (ATCC #TIB-47). After 2 days of co-culture the non-adherent BW5147 cells were removed and selected for resistance to puromycin. The puromycin resistant cell pool was subcloned by limiting dilution and tested for surface expression of human CTLA-4 by FACS. A clone expressing high levels of human CTLA-4 at the cell surface was selected (BW-huCTLA-4CD3.zeta.-3#12). Soluble recombinant antigen comprising the extracellular domain of human CTLA-4 was purchased from R&D Systems (Cat. #325-CT-200).

Description Paragraph (231):
Immunization. Three SC20/KCo5 cross-bred mice (ID#'s 22227, 22230, and 22231) were each immunized by intra-peritoneal (i.p.) injection of 10e7 washed whole BW-huCTLA-

4CD3.zeta.-3#12 cells expressing the human CTLA-4 extracellular domain. This immunization procedure was repeated two more times at approximately one month intervals for mice #22227 and 22230. At month 3, Mouse #22231 was given a third i.p. injection of whole washed cells, while mice #22227 and 22230 were each injected i.p. and subcutaneously (s.c.) with 20 micrograms of soluble recombinant antigen in MPL+TDM adjuvant (Sigma Cat. # M6536). The mice were then rested for 10 days and then two days prior to harvesting of spleen cells for hybridoma fusion, mice # 22227 and 22230 were each given tail vein (i.v.) injections of 20 micrograms soluble recombinant antigen together with i.p. injections of 20 micrograms of soluble recombinant antigen in MPL+TDM adjuvant. One day prior to harvesting splenocytes, these mice were given an additional i.v. injection of 20 micrograms of soluble recombinant antigen. Mouse #22231 was given 10e7 washed BW-huCTLA-4CD3.zeta.-3#12 cells in MPL+TDM adjuvant i.p. three days prior to harvesting spleen cells, followed by 10.sup.7 washed BW-huCTLA-4CD3.zeta.-3#12 cells without adjuvant i.p. two days prior to fusion.

Description Paragraph (232):
Fusion. Spleen cells from mice #22227, 22230, and 22231 were fused, in three separate experiments, with mouse myeloma cells (line P3 X63 Ag8.6.53, ATCC CRL 1580, or SP2/0-Ag14, ATCC CRL 1581) by standard procedures (Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y.; Kennett et al., 1980, Monoclonal Antibodies, Hybridomas:A New Dimension in Biological Analysis. Plenum, New York; Oi and Herzenberg, 1980, Immunoglobulin Producing Hybrid Cell Lines, in SELECTED METHODS IN CELLULAR IMMUNOLOGY, ed. Mishell and Shiigi, pp. 357 372. Freeman, San Francisco; Halk, 1984, Methods in Enzymology:Plant Molecular Biology, ed. Weissbach and Weissbach, pp. 766 780, Academic Press, Orlando, Fla.). Cells were cultured in DMEM, 10% FBS, OPI (Sigma O-5003), BME (Gibco 21985-023), and 3% Origen Hybridoma Cloning Factor (Igen IG50-0615). HAT or HT supplement was added to the medium during initial growth and selection.

Description Paragraph (233):
Hybridoma Screening. To identify hybridomas secreting antigen reactive human IgG antibodies, ELISA plates (Nunc MaxiSorp) were coated overnight at 4.degree. C. with 100 I/well Human CD152 Mu-Ig fusion (Ancel # 501-820) at 0.2 .mu.g/ml in PBS. Plates were washed and blocked with 100 .mu.l/well PBS-Tween containing 1% BSA. Fifty .mu.l of cell culture supernatant was added followed by a 1 2 hour incubation. Plates were washed and then incubated for one hour with 100 .mu.l/well goat anti-human gamma heavy chain conjugated to alkaline phosphatase (Anti-human gamma (fc) AP Jackson # 109-056-098). Plates were washed three times in PBS-Tween between each step. Seventy six hybridomas were identified that secreted gamma positive, antigen reactive antibody. These clones were then further analyzed to determine the gamma heavy chain or light chain isotype, as well as the presence of contaminating IgM secreting cells (Table 3).

Description Paragraph (234):
TABLE-US-00005 TABLE 3 Analysis of heavy chain isotypes from 1.degree. hybridoma wells comprising antigen reactive human IgG antibodies. Mouse ID # IgM IgG.sub.1 IgG.sub.2 IgG.sub.3 IgG.sub.4 Ig.kappa. Ig.lamda.- All IgG 22227 0 4 1 0 3 7 0 8 22230 9 25 8 5 7 48 6 45 22231 1 11 2 3 7 23 1 23 total 10 40 11 8 17 75 7 76

Description Paragraph (235):
Hybridoma supernatants were first tested for the presence of antigen reactive human IgG. Seventy six positive supernatants were then tested for antigen reactive human IgM, IgG.sub.1, IgG.sub.2, IgG.sub.3, IgG.sub.4, Ig.kappa., and mouse Ig.lamda.. Capture reagent:human CD152 mu-Ig fusion (Ancel # 501-820). Detecting reagents:anti-human gamma (fc) HRP (Jackson # 109-036-098); anti-human kappa HRP (Bethyl # A80-115P); anti-human gamma 1 HRP (Southern Biotech #9050-05); anti-human gamma 2HRP (Southern Biotech #9070-05); anti-human gamma 3HRP (Southern Biotech #9210-05); anti-human gamma 4HRP (Southern Biotech #9200-05); anti-human mu HRP

(Southern Biotech #1060-05).

Description Paragraph (236):
Seventy five of the 76 IgG antigen positive wells were also positive for human kappa light chain antigen reactive antibody, while 7 of the wells were positive for mouse lambda containing hybrid antibody. However, 6 of the 7 lambda positive wells also contained kappa light chain, and 3 of these three wells were positive for contaminating IgM antigen reactive antibody. Because these contaminating IgM antibodies may have contributed include the lambda light chain, there are between 3 and 7 IgG.lamda. clones out of the total of 76 IgG clones. Thus, the endogenous mouse lambda appears to contribute to only 4 to 9% of the IgG positive, antigen reactive hybridomas. Cells from 22 of the 76 positive hybridoma wells were then re-plated at limiting dilution to subclone individual monoclonal antibody secreting hybridomas. Stable antigen reactive, human IgG subclones were obtained from 19 out of 22 of the 1.degree. hybridomas (see Table 4 below).

Description Paragraph (237):
TABLE-US-00006 TABLE 4 Subcloning of Anti-CTLA-4 Hybridomas # Clones # % Clone OD Tested Positive Positive 4C1 0.44 24 5 21% 2E4 1.48 24 9 38% 1H5 1.39 24 14 58% 9C4 1.30 24 5 21% 6D11 3.24 16 10 63% 10H3 1.59 16 2 13% 8H4 3.14 16 7 44% 8G5 1.38 8 3 38% 4A9 1.35 24 20 83% 10E1 1.17 24 3 13% 9F6 1.08 24 0 0% 6B9 1.16 16 5 31% 9B10 2.70 32 9 28% 10D1 0.90 48 6 13% 1B6 1.34 24 9 38% 4C7 1.34 8 2 25% 1D11 0.97 8 0 0% 1B5 2.75 8 3 38% 4E9 1.36 24 1 4% 11H7 0.40 16 0 0% 2D8 1.31 24 10 42% 8F2 1.28 16 5 31%

Description Paragraph (238):
Thus 86% subcloning efficiency was obtained. On subcloning, it was found that one of the 1.degree. hybridomas comprised 2 distinct clones, having different IgG isotypes (see Table 5 below).

Description Paragraph (241):
Monoclonal antibodies were isolated from five of the subcloned hybridomas (1H5, 4A9, 4C1, 8H4, and 10E1) and tested for their ability to block CTLA-4 binding to B7.2 (FIGS. 12 and 13).

Description Paragraph (246):
In addition to linking to latex microspheres or other insoluble particles, the antibodies can be cross-linked to each other or genetically engineered to form multimers. Cross-linking can be by direct chemical linkage, or by indirect linkage such as an antibody-biotin-avidin complex. Cross-linking can be covalent, where chemical linking groups are employed, or non-covalent, where protein-protein or other protein-ligand interactions are employed. Genetic engineering approaches for linking include, e.g., the re-expression of the variable regions of high-affinity IgG antibodies in IgM expression vectors or any protein moiety (e.g., polylysine, and the like). Converting a high affinity IgG antibody to an IgM antibody can create a decavalent complex with very high avidity. IgA.sub.2 expression vectors may also be used to produce multivalent antibody complexes. IgA.sub.2 can form polymers together with J chain and secretory component. IgA.sub.2 may have the added advantage that it can be additionally crosslinked by the IgA receptor CD89, which is expressed on neutrophils, macrophages, and monocytes. Alternatively, because approximately 2% of the hybridomas generated from the C20/KCo5 cross-bred mice are IgA, these animals can be used to directly generate a human IgA isotype anti-CTLA-4 antibody.

Description Paragraph (254):
Hybridoma Screening. Screening procedures for EGFR hybridomas were similar to those used for the CTLA-4 in Example 8. ELISA plates (Nunc MaxiSorp) were coated overnight with 100 .mu.l per well of soluble EGFR antigen at 1 .mu.g/ml in PBS. Plates were washed and blocked with 100 .mu.l/well PBS-Tween containing 1% BSA. Fifty .mu.l of cell culture supernatant was added followed by a 1 2 hour

incubation. Plates were washed and then incubated for one hour with 100 .mu.l/well goat anti-human gamma heavy chain conjugated to alkaline phosphatase (Anti-human gamma (fc) AP Jackson # 109-056-098). Plates were washed three times in PBS-Tween between each step. Five and two hybridomas secreting human IgGic anti-EGFR specific antibodies were subcloned from the mouse 22232 and the mouse 22239 fusions respectively. Isotype analysis of the heavy and light chains of the EGFR specific antibodies included four IgG.sub.1.kappa., one IgG.sub.2.kappa. and one IgG.sub.4.kappa. antibodies.

Description Paragraph (257):
The hybridomas were cultured in eRDF containing 1% Fetal Bovine Serum (low-IgG). Human MAbs were purified using Protein G column. The rate equilibrium association constants of the purified MAbs for G-CSF and soluble CD4 were determined using BIAcore2000 instrument. Human G-CSF (120 RU) or CD4:Fc (1600 RU) was immobilized by covalent coupling through amine groups to the sensor chip surface of a BIAcore2000 (BIAcore) according to manufacture's instructions. The monoclonal antibody was flowed over the antigens. The chip was regenerated with Glycine-HCl buffer (PH1.5) or 4M MgCl.sub.2 to remove any residual anti-human G-CSF MAb or anti-CD4 MAb, respectively. This cycle was repeated, using different concentration of MAb. The binding to and dissociation from antigen were determined using BIAevaluation 3.0 software. The Ka was derived by dividing the k.sub.assoc by the k.sub.dissoc. As shown in Table 6 below, these values are comparable to those obtained for the murine anti-human G-CSF MAb, clone 3316.111 (R&D), or murine anti-CD4 MAb, Leu3a (Pharmingen)

Description Paragraph (262):
As described below, the Fc.gamma.RIIB mutation was bred into cross-bred mice of the invention. Immunization of the resultant cross-bred(Fc) mice with bovine C-IV elicited in human antibody responses against both bovine and murine C-IV. Hybridomas secreting human monoclonals that bind to both bovine and murine C-Iv can also be generated. Therefore, the cross-bred(Fc) mice allow for the production of human monoclonals that can bind both immunized foreign antigens and their murine counterparts. The cross-bred(Fc) mice can also be useful for obtaining human monoclonal antibodies against well-conserved antigens. Mice homozygous for the Fc.gamma.RIIB-knockout (Fc(-/-)) (Takai, T. et al., 1996, Nature 379:346 349) were provided by Dr. Toshifumi Takai (Tohoku University, JAPAN). The Fc(-/-) male mice were mated with female cross-bred mice (as described in Example 3). The retention of the KCo5 transgene and hCF(SC20) in each F1 individual was examined by ELISAs and PCRs as described in Example 3. Genotypes of Fc.gamma.RIIB-knockout were determined by PCR analysis using the three primers as follows:

Description Paragraph (269):
Fusion and Hybridoma Screening. The mice were given an additional intraperitoneal (KM#1: cross-bred, FC#1: cross-bred(Fc)) or intravenous (KM#2: cross-bred, FC#2: cross-bred(Fc)) injections of 150 .mu.g of antigen 66 days later and spleen cells were harvested 69 days later. Spleen cells from mice were fused with mouse myeloma cells (Sp2/0-Ag14) by standard procedures. The cell suspension were inoculated into 96-well plates at 200 thousands of splenocytes per well. Cells were cultured in DMEM, 10% FBS, Insulin, IL-6. HAT or HT supplement was added to the medium during initial growth and selection. The hybridomas were screened by ELISA. To identify hybridomas secreting mouse C-IV, ELISA plates (Nunc MaxiSorp) were coated overnight at 4 degree with 50 .mu.l/well mouse C-IV (Sigma, C0534) at 40 .mu.g/ml in PBS. Fifty .mu.l of cell culture supernatant was added. Two hybridomas secreted h.gamma. positive, mouse C-IV reactive antibody were obtained from a cross-bred(Fc) mice and were successfully subcloned by limiting dilution (see Table 7 below).

Other Reference Publication (115):
Szurek, P., et al., "Complete nucleotide sequence of the murine .gamma.3 switch region and analysis of switch recombination in two .gamma.3-expressing hybridomas", J. Immunol. 135:620-626 (1985). cited by other

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